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UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 844

Contribution from the Bureau of Plant Industry
WM. A. TAYLOR, Chief

Washington, D. C. PROFESSIONAL PAPER August 11, 1920

SWEET-CLOVER SEED

Part I.—Pollination Studies of Seed Production

Part II.—Structure and Chemical Nature of the Seed Coat and its Relation to Impermeable Seeds of Sweet Clover

By

H. S. COE, formerly Assistant Agronomist, Office of Forage-Crop Investigations, and J. N. MARTIN, Professor of Morphology and Cytology, Iowa State College

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Part I.—POLLINATION STUDIES OF SEED PRODUCTION.

UNSATISFACTORY YIELDS OF SWEET-CLOVER SEED.

In some sections of the country much trouble has been experienced for a few years past in obtaining satisfactory yields of sweet-clover seed. This difficulty has been due for the most part to the following causes: (1) To cutting the plants at an improper stage of develop-

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ment, (2) to the use of machinery not adapted to the handling of the crop, (3) to the shedding of immature pods, and (4) possibly to the lack of pollination. As the first two have been overcome, mainly because of a better understanding of the requirements for handling this crop, the subject matter of this bulletin is concerned primarily with the factors which produce the third and fourth causes.

Where the production of seed was disappointing although the plants produced an abundance of flowers, it has been observed that many apparently were not fertilized, or if fertilized the pods aborted. In order to obtain data in regard to the causes of the failure of sweet clover to produce a normal seed yield, a study was made of the insects which were most active in pollinating the flowers, the source of the pollen necessary to effect fertilization, and the conditions under which the flowers must be pollinated in order to become fertilized. The relation of environmental conditions to the shedding of immature pods was also investigated. In order to overcome local environmental factors as much as possible, the experiments were conducted on the Government Experiment Farm at Arlington, Va., and in cooperation with the botanical department of the Iowa State College at Ames, Iowa.

PREVIOUS INVESTIGATIONS OF THE POLLINATION OF SWEET CLOVER.

Since Darwin (4, p. 360)[1] published the statement that a plant of *Melilotus officinalis* protected from insect visitation produced but a very few seeds, while an unprotected plant produced many, other scientists have investigated this subject. Knuth (19, v. 1, p. 37), in giving a list of the best known cases of self-sterility in plants, mentions *Melilotus officinalis*. The same author (19, v. 2, p. 282) states that since the stigma projects beyond the anthers, automatic self-pollination is difficult, and for the same reasons Müller (29, p. 180) believes that self-fertilization is not apt to occur.

In 1901 Kirchner (18, p. 7) covered a number of *Melilotus alba* racemes with nets. On one of the plants 12 protected racemes produced 187 seeds and on another plant only one seed was obtained from 10 covered racemes. This experiment was duplicated in 1904, with the result that 40 netted racemes produced an average of 38 seeds each. Kirchner concluded from this experiment that spontaneous self-pollination occurs regularly even though the stigma projects above the anthers. He (18, p. 8) also performed an experiment with *Melilotus officinalis* in 1901. At this time 16 isolated racemes produced a total of 11 seeds. This experiment was repeated in 1904, with the result that 16 protected racemes produced an average of 14 seeds each. As the racemes on one of the plants that was protected

[1] The serial numbers in parentheses refer to "Literature cited," pages 36–38.

in 1904 died, Kirchner concluded that the flowers of *M. officinalis* were especially sensitive to inclosure in nets and that the failures to obtain more than a very few seeds on protected racemes in Darwin's experiment and in his first experiment were due to this cause.

According to Kerner (17, v. 2, p. 399) the peas and lentils (Pisum and Ervum) and the different species of horned clover and stone clover (Lotus and Melilotus) as well as the numerous species of the genus Trifolium and also many others produce seeds when insects are excluded from the plants, and only isolated species of these genera gave poor yields without insect visitation.

OUTLINE OF POLLINATING EXPERIMENTS.

The yield of sweet-clover seed varies greatly from year to year in many parts of the United States. It has been assumed that this variation was due to climatic conditions, as excellent seed crops were seldom harvested in seasons of excessive rainfall or of prolonged drought just preceding or during the flowering period. The lack of a sufficient number of suitable pollinating insects also was thought to be an important factor in reducing seed production. This was especially true where the acreage of sweet clover was large and where few, if any, honeybees were kept.

In order to obtain data upon the factors influencing the yield of seed, a series of experiments was outlined to determine (1) whether the flowers are able to set seed without the assistance of outside agencies, (2) whether cross-pollination is necessary, (3) the different kinds of insects which are active agents in pollinating sweet clover, and (4) whether a relation exists between the quantity of moisture in the soil and the production of seed.

The racemes containing the flowers which were to be pollinated by hand were covered with tarlatan before any of the flowers opened and were kept covered except while being pollinated until the seeds were nearly mature. This cloth has about twice as many meshes to the linear inch as ordinary mosquito netting and served to exclude all insects that are able to pollinate the flowers. When entire plants were to be protected from all outside agencies, cages covered with cheesecloth, glass frames, or wire netting were used.

A preliminary study of the pollination of *Melilotus alba* and *M. officinalis* showed that both were visited by the same kinds of insects and that both required the same methods of pollination in order to set seed. On this account *M. alba* was used in most of the experiments reported in this bulletin. Where *M. officinalis* was employed it is so stated.

STRUCTURE OF THE FLOWERS OF MELILOTUS ALBA.

The racemes of *Melilotus alba* contain from 10 to 120 flowers with an average of approximately 50 per raceme for all of the racemes of a plant growing under cultivation in a field containing a good stand.

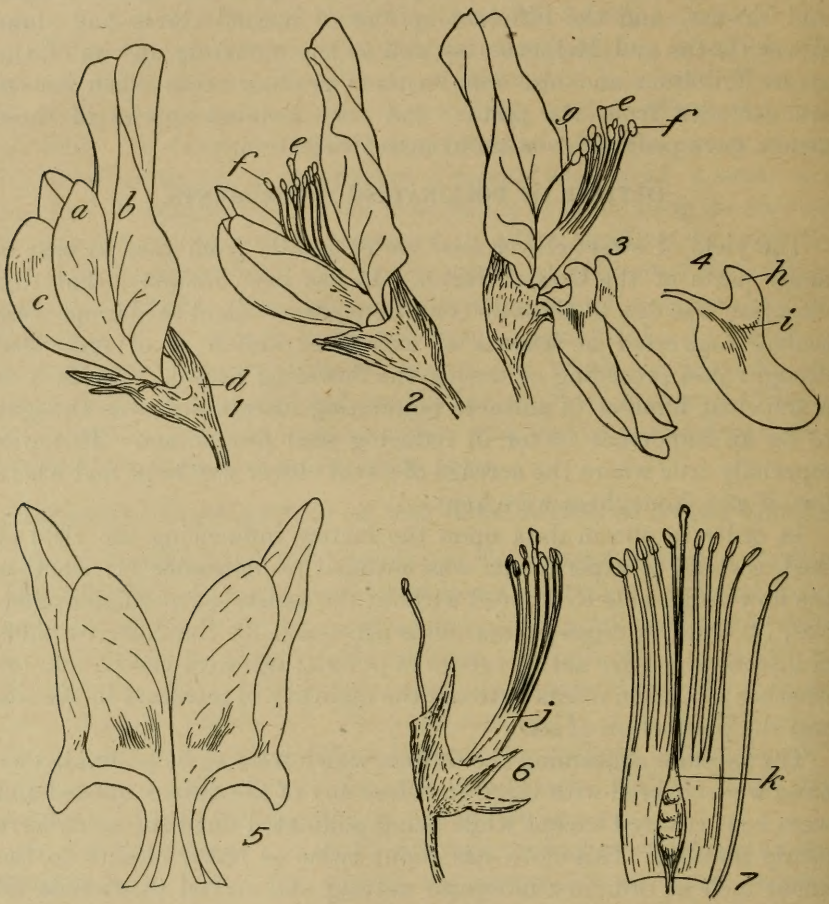


FIG. 1.—Different parts of the flower of *Melilotus alba:* 1, Side view of the flower; 2, side view of the flower with the carina and alæ slightly depressed; 3, side view of the flower, showing the carina and alæ depressed sufficiently to expose the staminal tube and the tenth free stamen; 4, ala; 5, alæ and carina spread apart to show their relative position and shape; 6, flower after the petals have been removed, showing in detail the calyx and staminal tube; 7, the staminal tube split open to show the relative size and position of the pistil. *a*, Alæ; *b*, vexillum; *c*, carina; *d*, calyx; *e*, stigma; *b*, anthers; *g*, tenth free stamen; *h*, digitate process of the superior basal angle of an ala; *i*, depressions in the ala; *j*, staminal tube; *k*, pistil.

The flower consists of a green, smooth, or slightly pubescent calyx with 5-pointed lobes and with an irregular white corolla of five petals. (Fig. 1.) The claws of the petals are not united nor are they attached to the staminal tube which is formed by the union of the filaments of the nine inferior stamens. As the claws of the alæ and carina are not

attached to the staminal tube, the petals may be bent downward sufficiently far so that many different kinds of insects may secure without difficulty the nectar secreted around the base of the ovary.

The fingerlike processes of the alæ are appressed closely to the carina, therefore the alæ are bent downward with the carina by insects. These processes grasp the staminal tube superiorly and tightly when the carina and alæ are in their natural positions, but when the carina is pressed downward by insects the fingerlike processes open slightly but not so far that they do not spring back to their original position when the pressure is removed. The staminal tube splits superiorly to admit the tenth free stamen. The filament of this superior stamen lies along the side of this staminal tube. The filaments of the nine stamens which compose the staminal tube separate in the hollow of the carina. All stamens bear fertile anthers. The pistil is in the staminal tube, the upper part of the style and stigma of which is inclosed with the anthers in the carina. The stigma is slightly above the stamens.

An insect inserts its head into a sweet-clover flower between the vexillum and carina, the stigma, therefore, comes into direct contact with the head of the insect and cross-pollination is effected. At the same time the anthers brush against the insect, so that its head is dusted with pollen, to be carried to other flowers.

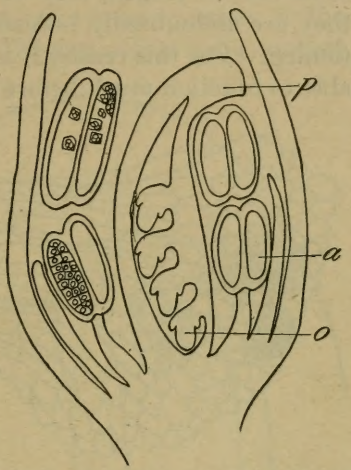


FIG. 2.—Lengthwise sectional view of a very young flower of *Melilotus alba*, showing the relative development of the stamens and pistil. In the upper set of stamens the divisions of the mother cells are completed, while division is just beginning in the lower set of stamens. In the ovules the outer integuments are well started on their development. *a*, Anther; *o*, ovule; *p.*, pistil. ×38.

DEVELOPMENT OF THE FLORAL ORGANS OF SWEET CLOVER.

The stamens of *Melilotus alba* and *M. officinalis* may be divided into two sets, according to their length and time of development. (Fig. 2.) The longer set extends about the length of the anthers above the shorter set, and the pollen mother cells in the longer set divide to form pollen grains at least two days earlier than those in the shorter set. At the time the pollen mother cells divide, the longer set of stamens is approximately three-eighths of a millimeter in length and the pistil about half a millimeter long. The stigma and a portion of the style project beyond the stamens, and this relative position is maintained to maturity. The pollen mother cells undergo the reduction division while the megaspore mother cells are

just being differentiated and while the outer integuments are barely prominent at the base of the nucellus. The pollen grains are formed while the embryo sac is beginning to develop. The division of the megaspore mother cell does not occur until a number of days later, and the embryo sac is not mature until the flower is nearly ready to open. Thus, the pollen grains are formed a week to 10 days before the embryo sac is ready for fertilization. The pollen grains increase in size and undergo internal changes after their formation. These changes, which are not completed until the flower is one-half or more of its mature length, may be regarded as the ripening processes, and they are undoubtedly necessary before the pollen is capable of functioning. For this reason it is probable that the pollen grains are not able to function much before the embryo sac is mature.

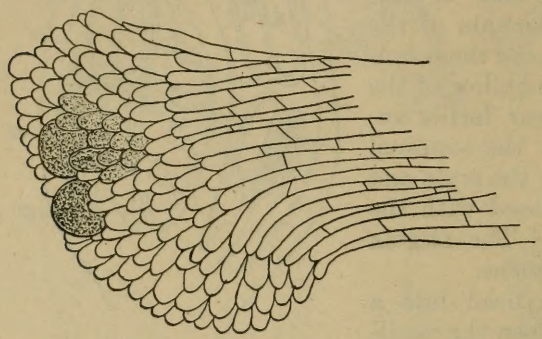


FIG. 3.—Stigma at the time of pollination, showing its papillate character and the position of the pollen in reference to the papillæ in pollination. ×175.

The pistils of *Melilotus alba* and *M. officinalis* are straight for the greater part of their length, but curve rather abruptly toward the keel just below the capitate stigma. The surface of the stigma is papillate. (Fig. 3.) In their reaction with Sudan III, alkanin, and safranin the walls of the papillæ of the stigma show that some fatlike substances are present. Aside from water, the contents of the papillæ consist chiefly of a fine emulsion of oil.

DEVELOPMENT OF THE OVULES.

The number of ovules in the ovary of *Melilotus alba* varies from two to five; however, most commonly, three or four ovules occur. In *Melilotus officinalis* the number in each ovary ranges from three to six. In both species the ovules are campylotropous at maturity with the micropylar end turned toward the base of the ovary.

Mature ovules contain two integuments, but the inner one does not close entirely around the end of the nucellus. The outer integument develops considerably ahead of the inner one. The outer integument is much thickened at the micropylar end, the seed coat is formed from it, and the inner integument is used as nourishment by the endosperm and embryo.

The number of megaspore mother cells in an ovule varies from one to many. Two or more embryo sacs often start to develop in the same ovule, but seldom more than one matures. (Pl. I, figs. 1,

2, and 3.) In general, the development of the embryo sac proceeds in the ordinary way, as described by Young (44, p. 133), with the inner megaspore functioning. (Text fig. 4 and Pl. II, fig. 1.) In its development the nucellus is destroyed rapidly, the destruction being most rapid first at the micropylar end proceeding backward. The nucellus is completely destroyed at the micropylar end by the time the embryo sac is mature, and consequently the embryo sac comes in contact with the outer integument in this region. (Pl. II, fig. 1.) As the destruction of the nucellus extends toward the chalazal end the embryo sac becomes much elongated and tubelike. The antipodals disappear early, so that a mature embryo sac consists of the egg, the synergids, and the two polars. The two polars lie in contact in the micropylar end of the sac near the egg until fertilization.

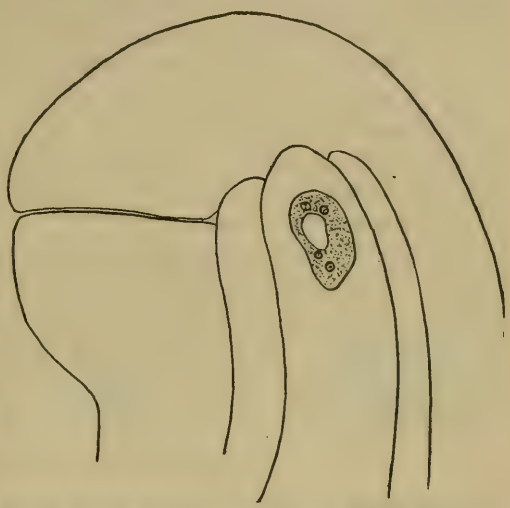


FIG. 4.—Median section through an ovule, showing the embryo sac with four nuclei and the position of the integuments. ×150.

STERILITY OF THE OVULES.

In *Melilotus alba* and *M. officinalis* there is very little tendency toward sterility of ovules. In an extended study of ovules developing under normal and under excessive moisture conditions only an occasional one was found in which no reproductive cells were differentiated, and no ovaries were found in which all of the ovules were sterile.

DEVELOPMENT OF THE POLLEN.

The pollen mother cells do not separate, but previous to the reduction division the protoplasm shrinks from the walls, thus forming a dense globular mass which often occupies less than half the lumen of the mother cell. (Pl. I, fig. 4.) Nuclear division occurs while they are in this contracted condition, and four nuclei are formed from two successive divisions. The cytoplasm is equally distributed around each nucleus. The four masses of protoplasm separate, and as they enlarge a number of times and develop into mature pollen grains they become binucleate, and a wall is gradually formed around each. (Pl. I, figs. 5 and 6.) At first the cytoplasm is quite dense and contains some starch but no fatty oils. However, the cytoplasm of

mature pollen grains is vacuolate, and it contains a fatty oil in the form of an emulsion. Soon after the pollen grains are formed, the walls of the mother cells disappear, thus permitting the pollen grains to lie loose in the anther.

FERTILIZATION IN MELILOTUS ALBA.

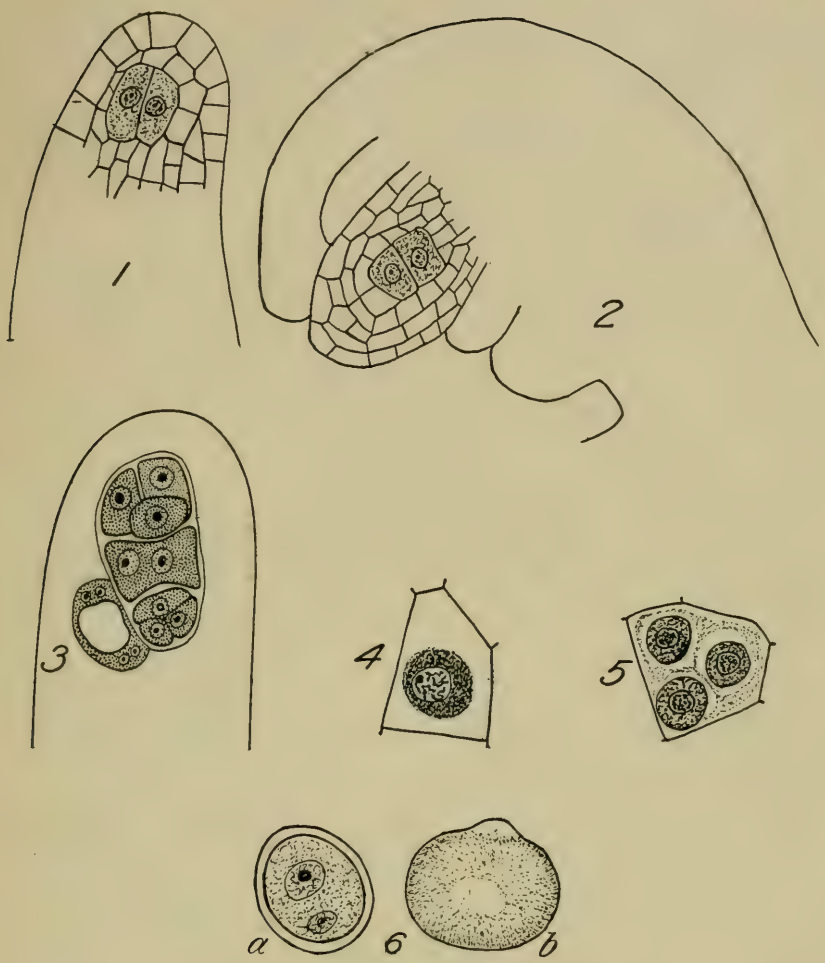
The time intervening between pollination and fertilization was investigated with both self-pollinated and cross-pollinated flowers. In cross-pollination the parents were separate plants. This point was investigated with plants out of doors during the summer of 1916 and with plants in the greenhouse during the following winter. The time elapsing between pollination and fertilization ranged from 50 to 55 hours and was not longer in the case of self-pollinated than with cross-pollinated flowers. Furthermore, the rate of the development of the embryo in each kind of pollination was studied and was found to be as rapid in self-pollination as in cross-pollination. Therefore, self-pollination is apparently as effective as cross-pollination in *Melilotus alba* so far as the vigor of pollen tubes and the rate at which embryos develop are concerned. *Melilotus officinalis* was not studied in reference to this point.

Considerable difference often exists in the size of the young embryos in the ovules of the same pod. This is due in part to a difference in the time of fertilization, although some of it is due to a difference in nourishment. It was observed that the ovule first fertilized might be an upper one, lower one, or any one between these. Occasionally one or more ovules are not fertilized.

DEVELOPMENT OF THE SEED.

A proembryo with a rather long suspensor is developed from the fertilized egg. (Pl. II, fig. 2.) The endosperm, which quite early forms a peripheral layer around the entire embryo sac, develops most rapidly about the embryo, which soon becomes thoroughly embedded in it. (Pl. III, figs. 1 and 2.) After the embryo has used up the endosperm in the micropylar end and has enlarged so much as to occupy nearly all of the space in this region, the development of the endosperm becomes more active in the chalazal end, and when the embryo is mature there is very little endosperm left.

The seed coat begins to form about the time of fertilization, although it apparently does not depend upon it, for in ovules where fertilization is prevented the outer integument undergoes the early modifications in the development of the seed coat before the ovule breaks down. The development of the seed coat is apparent first at the micropylar and chalazal ends, where the outer cells of the outer integument become much elongated and their outer walls thicken very soon after fertilization. The modifications in the development



DEVELOPMENT OF THE OVULES AND POLLEN IN SWEET CLOVER.

FIG. 1.—Section through the nucellus of an ovule of *Melilotus alba*, showing two megaspore mother cells. ×360. FIG. 2.—Median section through an ovule of *Melilotus alba*, showing the two cells resulting from the first division of the megaspore mother cell, and the relative development of the different parts of the ovule. ×300. FIG. 3.—Section through the nucellus of an ovule of *Melilotus alba*, showing two embryo sacs, one being more advanced than the other. ×360. FIG. 4.—Protoplasm of the pollen mother cell of *Melilotus alba* contracted and ready to undergo division. ×560. FIG. 5.—Pollen grains of *Melilotus alba* just formed, showing their dense cytoplasm and the presence of the mother-cell wall. ×560. FIG. 6.—*a*, Mature pollen grain of *Melilotus alba*, showing the binucleate condition at the time of shedding; *b*, surface view. ×560.

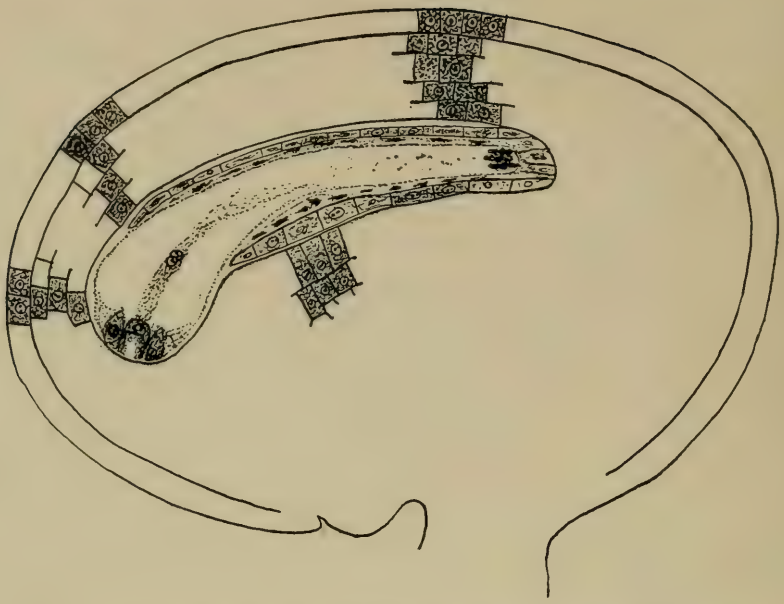


FIG. 1.—MEDIAN SECTION THROUGH AN OVULE OF MELILOTUS ALBA.

The embryo sac is shown ready for fertilization. The egg and synergids are in contact with the outer integument at the micropylar end. The remains of the antipodals may be seen at the chalazal end. ×180.

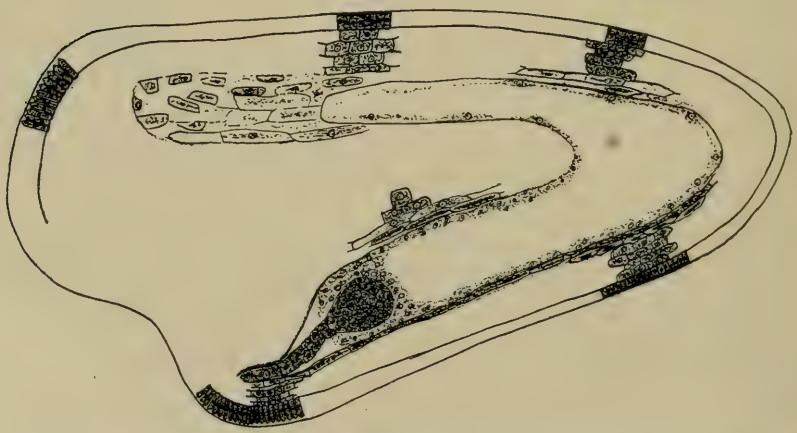


FIG. 2.—SECTION THROUGH AN OVULE OF MELILOTUS ALBA, ABOUT THREE DAYS AFTER FERTILIZATION.

The proembryo, the endosperm, and modifications of the integuments are shown. At this stage the suspensor is a prominent part of the proembryo, and the endosperm is most abundant around the embryo. The inner integument is being rapidly destroyed, and the outer integument is beginning to form the seed coat, as is indicated by the modifications in the outer layer of its cells, which are elongating and thickening their outer walls. ×33.

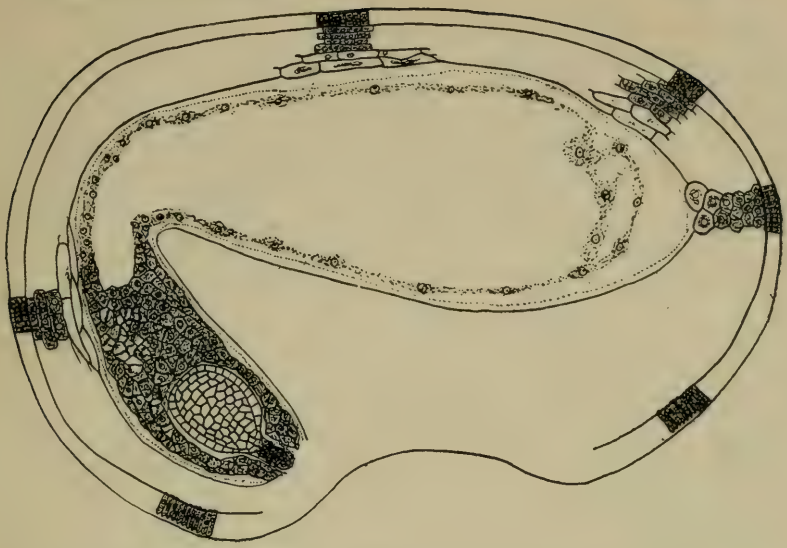


FIG. 1.—SECTION OF AN OVULE OF MELILOTUS ALBA AFTER FERTILIZATION.

The stage of development is a little later than that shown in Plate II, figure 2. The embryo is embedded deeply in endosperm tissue. ×45.

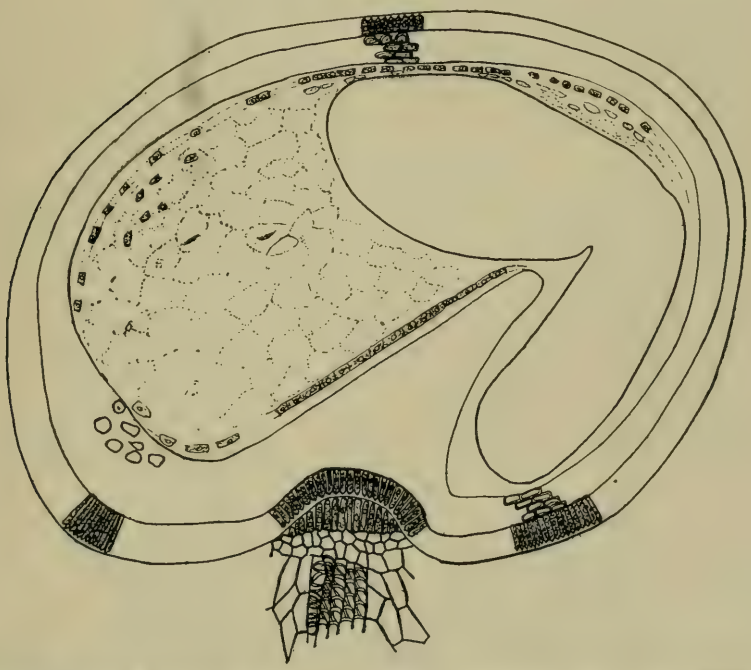
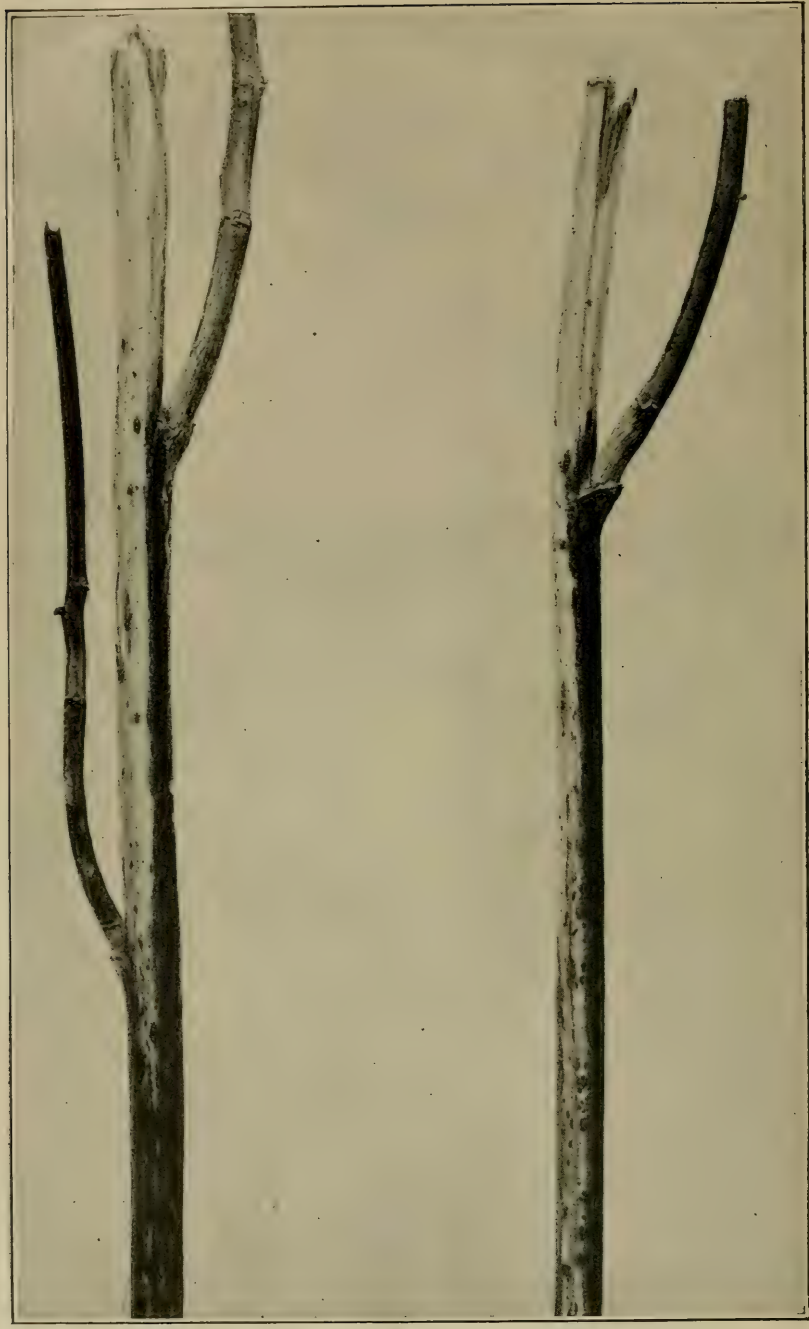


FIG. 2.—SECTION THROUGH AN OVULE OF MELILOTUS ALBA AFTER THE EMBRYO IS NEARLY HALF MATURE.

But little endosperm remains except in the chalazal end, and very little remains of either the nucellus or inner integument. The modifications which transform the outer integument into a seed coat are well under way. Not only the outer layer of cells which becomes the Malpighian layer is quite well modified, but also the layer beneath is being transformed into the osteosclerid layer. ×30.



STUBBLE OF MELILOTUS ALBA.

These plants, which were cut 12 inches above the ground during rainy weather, had made a 40 to 42 inch growth. The stubble became infected at the top and the light-colored portions of them were killed by disease, thus checking the water supply to the growing branches above the infection.

of the seed coat extend around the ovule from these points, involving at first only the outer or epidermal layer of cells which form the malpighian layer. Later, the cells just beneath the malpighian layer form the osteosclerid layer. Accompanying or closely following the formation of the osteosclerid cells, the remaining cell layers of the outer integument become modified into the nutritive and aleurone layer, and the seed coat is fully formed. Meantime the inner integument is practically all used as food.

MATURE POLLEN OF SWEET CLOVER.

The pollen grains of *Melilotus alba* and of *M. officinalis* are quite similar. Each grain contains three germ pores, and when viewed so that the pores are visible they present a slightly angled appearance. The average dimensions of the pollen of *Melilotus alba* and of *M. officinalis* are 26 by 32 microns and 24 by 30 microns, respectively, when measured in the positions shown in *b* in Plate I, figure 6.

The walls of the pollen grains have cutin deposited in them, as shown by their reactions with Sudan III, alkanin, safranin, and chloriodid of zinc. The contents of the pollen grains give a distinct reaction when tested for fat, and Millon's reagent shows that also some protein is present. Tests for sugars and starch showed that these substances are not present in perceptible quantities in mature pollen grains, although some starch is present in immature pollen.

GERMINATION OF THE POLLEN.

The germination of the pollen of *Melilotus alba* permits considerable variation in moisture, as is illustrated in Table I.

TABLE I.—*Germination of the pollen of Melilotus alba in water and in solutions of cane sugar of different strengths.*

Melilotus alba.	Pure water.	Cane sugar in solution (per cent).							
		8	12	18	24	30	35	45	55
Germination of pollen.....per cent..	33	23	64	46	60	46	31	22	0

The results given in Table I represent the average of 12 tests. Some of the pollen grains burst in pure water and in the weak cane sugar solutions, the percentage of bursting being greatest in pure water and decreasing as the percentage of sugar in the solution was increased. There was considerable variation in the percentages of germination in both water and in the solutions of different strengths, and at times there was very little bursting which was not accompanied by a high percentage of germination. The pollen tubes grew as rapidly in water as in any of the sugar solutions, some reaching a

length of 100 microns in six hours. As the pollen tubes made no more growth in the solutions of sugar than in water, it is evident that the sugar is not used as food, but helps in germination by reducing the rate at which water is absorbed.

To judge from Table I, the pollen of sweet clover can be effective not only under ordinary conditions but also when the flowers are wet with rain or dew or when the stigma is so dry that in order to obtain water from the papillæ the pollen must overcome a high resistance offered by the sap of the papillæ, a resistance that may be equal to the osmotic pressure of a 45 per cent solution of cane sugar. This is in accord with results obtained under field conditions, as flowers that were pollinated while rain was falling set seed satisfactorily, indicating that a high percentage of humidity in the atmosphere does not check the germination of the pollen sufficiently to interfere with fertilization. Neither was the setting of seed affected when the soil about the roots of plants was kept saturated with water, showing that the excessive quantity of water in the stigmas resulting from an abundance of water in the soil did not interfere with the fertilization of the flowers.

No definite counts were made of the germination of the pollen of *Melilotus officinalis* in the solutions of cane sugar of different strengths, but observations show that the moisture requirement of the pollen of this species is approximately the same as that of *Melilotus alba*.

CROSS-POLLINATION AND SELF-POLLINATION OF SWEET CLOVER.

Results published by previous investigators on the cross-pollination and self-pollination of sweet clover do not agree. The experiments of Darwin (4) show that the flowers are self-pollinated to only a small extent. On the other hand, Kirchner (18) and Kerner (17) find that self-pollination occurs generally and that cross-pollination is not necessary for the production of seed. However, all investigators agree that many different kinds of insects are able to pollinate sweet clover.

Because of the diverse opinions as to the pollination of sweet clover, a number of experiments were conducted to determine (1) whether insect visitation was necessary to pollinate the flowers, (2) if necessary, whether the flowers must be cross-pollinated, and (3) what insects are active agents as pollinators of sweet clover.

ARTIFICIAL MANIPULATION OF SWEET-CLOVER FLOWERS.[1]

Experiments were conducted to determine, if possible, the effect of various types of artificial manipulation of sweet-clover flowers when in full bloom on the production of seed. Only healthy, vigor-

[1] The writers wish to acknowledge their indebtedness to Mr. Carl Kurtzweil for assistance in conducting part of the field experiments at Ames.

ous plants growing on well-drained soil were selected for these experiments. Before any of the flowers were open, the individual racemes were covered with tarlatan and labeled. (Fig. 5.) As soon as part of the flowers opened, the racemes were uncovered and after removing all flowers that were not open the open flowers were pollinated and the racemes re-covered. If the flowers of sweet clover are not fertilized they will remain open for two to three days, then wither, and in a short time drop. But after being fertilized the ovules

FIG. 5.—Individual racemes of white sweet clover covered with cheesecloth to protect them from insect visitation.

enlarge very rapidly, and the corollas usually drop in about seven or eight days. Therefore, all fertilized flowers can be distinguished a few days after fertilization has taken place. Counts were made of the number of pods which formed in 10 to 12 days after pollination. An outline of the experiments is given in Table II.

TABLE II.—*Treatment of sweet-clover flowers in the artificial-manipulation experiments.*

Experiment.	Method of pollinating the flowers.
A	Check—covered.
B	Check—open to insect visitation at all times.
C	A separate toothpick was used to spring the keel of each flower on the raceme.
D	One toothpick was used to spring the keels of all the flowers on a raceme.
E	Cross-pollinated.
F	Raceme rolled several times between thumb and finger.

As insects, and especially honeybees, usually visit all recently opened flowers on a raceme, experiments C and D were conducted to determine whether more seed would be produced when pollen from other flowers on the same raceme was placed on the stigmas of the flowers than when only the pollen produced by each flower was placed on its own stigma. The effect of pollination when only the pollen produced by an individual flower was placed on its own stigmas was also obtained in experiment F, as by this method of pollination no pollen was transferred from one flower to another. It can not be stated definitely that the seed produced by the cross-pollinated flowers was the result of fertilization with foreign pollen, as the anthers were not removed from the flowers pollinated because it would be necessary to remove the anthers when the flowers were not more than two-thirds mature, and in doing this the flowers would be so mutilated that only occasionally would pollination at this time or at a later date be effective. The flowers listed in experiment E were pollinated a short time before they opened, and in each case pollen taken from flowers of other plants was placed on the stigmas. The petals of the cross-pollinated flowers were not mutilated, and in each case they returned to their original positions soon after pollination. The results obtained in experiment B, where the racemes were simply labeled and left open to the action of insects at all times, serve for comparison with other experiments where the flowers were protected from insect visitation and were artificially manipulated.

Martin (25) found the setting of alfalfa seed and Westgate (40) found the setting of red-clover seed to be affected by an excessive quantity of moisture in the soil or atmosphere. In order to overcome the possible effect of this or of other detrimental factors, in each experiment only the flowers on a certain number of racemes were pollinated at one time. All of the experiments were repeated a number of times during the months of July and August, 1916, and the results given in Table III show the total number of flowers pollinated and the number of pods that formed during the two months.

The results presented in Table III show that flowers fertilized with pollen transferred from another plant produced a higher percentage of pods than any of the other treatments. The results obtained in experiment D, where the same toothpick was used to spring the keels of all the flowers on a raceme, show that this method of pollination produced an average of 7.24 pods per raceme more than the racemes in experiment C, where a separate toothpick was used for each flower. These results indicate that pollen transferred from one flower to another on the same raceme is more effective than when the pollen produced by an individual flower is used to fertilize its own stigma. However, the results of experiment C prove that self-pollination is effective in *Melilotus alba*. In experiment B, which

was the open check, 4.3 per cent more flowers set seed than on the racemes where the same toothpick was used to spring all the keels, but 11.57 per cent more seed was obtained than in experiment C. Spontaneous self-pollination occurs to only a very small extent, as will be seen from the results of experiment A, in which an average of only 2.9 per cent of the flowers set seed.

TABLE III.—*Effect of different types of artificial manipulation on the seed production of sweet clover at Arlington, Va., and at Ames, Iowa, in 1916.*

Location.	Experiment.	Total number of—			Flowers that set seed (per cent).	
		Racemes.	Flowers.	Pods set.	At each station.	Average.
Arlington	A	49	3,510	144	4.1	2.9
Ames	A	84	4,536	92	2.0	
Arlington	B	100	5,599	3,973	70.95	66.51
Ames	B	196	1,276	600	47.02	
Arlington	C	50	1,229	701	57.03	54.94
Ames	C	75	289	133	46.02	
Arlington	D	50	1,480	936	63.24	62.18
Ames	D	88	575	342	59.47	
Arlington	E	31	377	307	81.43	70.10
Ames	E	48	175	80	45.71	
Arlington	F	30	933	524	56.16	.....

SEED PRODUCTION OF MELILOTUS ALBA UNDER ORDINARY FIELD CONDITIONS.

The production of seed of *Melilotus alba* under ordinary field conditions varies considerably, not only in different parts of the country but also on different fields in the same region. A number of factors contribute to this variation, one of the most important of which appears to be the inability of the plant to supply all the developing seed with sufficient moisture, causing some of them to abort. As pointed out on page 22 this condition was very marked in certain parts of the country in 1916. However, poor seed production is not always correlated with lack of moisture, for the seed crop was a failure in 1915, where cloudy and rainy weather prevailed much of the time the plants were in bloom. It is believed that the lack of pollination by insects was the principal cause for the failure of seed to set, as very few insects visit sweet-clover flowers when such conditions prevail. As sweet-clover pollen will germinate in pure water and as plants which have their roots submerged in water set seed abundantly when pollinated, the failure of the seed crop in 1915 was not due to excessive moisture.

As a rule, thin stands of sweet clover produce more seed to the acre than thick stands and isolated plants more seed than those growing in either a thick or thin stand. The correlation of seed

production with the thickness of stand is probably due to the shading and partial prevention of insect visitation to part of the racemes on the lower branches. Most of the flowers upon the lower branches of isolated plants are directly exposed to sunlight and to insect visits; therefore the racemes on these branches produce as large a percentage of seed as the racemes on the upper branches. In a thick stand, little seed is produced by racemes on the lower branches.

A plant approximately 3 feet high growing close to the center of a field at Arlington, Va., in which was an average stand of four sweet-clover plants to the square foot was selected in order to determine the number of racemes produced and the average number of seeds to the raceme. This plant produced 196 racemes, which contained an average of 20.4 pods each. The racemes varied from 2 to 10 cm. in length, and the number of pods to the raceme ranged from 0 to 75. The racemes on the upper and most exposed portions of the plants were larger and the flowers produced a much higher percentage of pods than the racemes close to the bases of the larger branches. Many of the small racemes on the lower branches produced less than five pods each.

The data obtained from the two plants at Arlington that were protected from night-flying insects may also be cited here, as the results of that experiment show that night-flying insects are not an important factor in the production of sweet-clover seed, and, further, because they were growing under the same conditions, in the same plat, and were approximately of the same size. These two plants produced a total of 544 racemes, with an average of 20.9 pods each. The number of pods to the raceme varied from 0 to 86.

EFFICIENCY OF CERTAIN KINDS OF INSECTS AS POLLINATORS OF SWEET CLOVER.

In order further to test the self-sterility of sweet clover and to determine the relative efficiency of night-flying and of different kinds of day-flying insects as pollinators of the flowers, a number of cages covered with cheesecloth, glass, or wire screen having 14 meshes to the linear inch were placed over plants at Arlington, Va., and at Ames, Iowa, in July and August, 1916. The bases of the cages were buried several inches in the ground, so that insects could not pass under them. Cheesecloth was used to cover most of the cages and was made into sacks of such a size that they could be put on or removed from the frames of the cages without difficulty. It was stretched tightly over the frames and fastened to their bases with laths.

A cage having two sides and the top of glass but with ends covered with cheesecloth to permit ventilation was used at Ames to protect a number of plants from insect visitation at all times. The purpose

of this cage was to determine whether the partial shading of the plants in the cages covered with cheesecloth would have any effect upon the setting of seed.

The cage covered with wire netting having 14 meshes to the linear inch was used to determine the efficiency as pollinators of sweet clover of insects so small that they could pass through openings of this size.

The plants used in the experiments at Arlington were growing close to the center of a field of sweet clover. Volunteer plants in a field that contained only a scattering stand were used at Ames. The cages were placed over the plants in all of these experiments before any of the flowers opened, and the work was continued until they were through blooming.

PLANTS SUBJECT TO INSECT VISITATION AT ALL TIMES.

A plant subject to insect visits at all times and growing in the same plat as those inclosed in the cages at Arlington was selected as a check to those inclosed in the cages during their entire flowering period or for only a portion of it. This plant, which was in bloom at the same time as those inclosed in the cages, produced 196 racemes with an average of 20.4 pods each. As all of the racemes were collected and as those on the lower portions of the plant were smaller than those on the upper branches, the average number of seeds per raceme is much lower than it would have been if only the larger racemes had been collected.

An isolated plant that was subject to insect visits at all times was selected for a check to the cage work conducted at Ames. This was necessary in order to get results that would be comparable with those obtained from the plants inclosed in the cages, as the cage experiments at Ames were conducted with isolated plants. The plant produced 239 racemes, with an average of 41.6 pods.

PLANTS PROTECTED FROM INSECT VISITATION DURING THEIR ENTIRE FLOWERING PERIOD.

On July 3, 1916, a cage 3 feet square and 3½ feet high, covered with cheesecloth, was placed over three sweet-clover plants at Arlington. (Fig. 6.) This cage was not opened until August 3, when practically all of the racemes had passed the flowering stage and the few seeds that formed on some of them were practically mature. The three plants inclosed in the cage produced 904 racemes, with an average of 0.63 pod each. No pods were produced on 594 racemes, while 150 produced but one each. None of the racemes produced more than five pods.

This experiment was duplicated at Ames in August, 1916, with the result that the three protected plants produced a total of 776 racemes, with an average of 0.19 pod each.

The plants inclosed at Arlington produced 0.44 pod to the raceme more than the plants inclosed at Ames, and the average for the six plants at Arlington and at Ames is only 0.42 pod to the raceme. Results given below for nine plants inclosed in the glass-covered cage show that the pods produced per raceme by different plants varied from 0.1 to 0.45, which is slightly less than the variation in the two cages covered with cheesecloth.

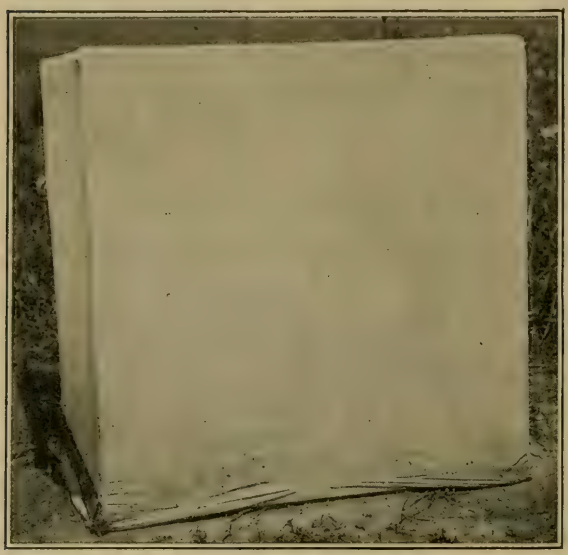


FIG. 6.—Cage covered with cheesecloth to protect plants from insect visitation.

In order to determine whether the shading of the plants in the cheesecloth-covered cages had caused the production of seed to be reduced, a cage 4 feet wide, 4 feet high, and 10 feet long, having glass sides and top, but with ends covered with cheesecloth to permit ventilation, was placed over nine plants at Ames in August, 1916. The results obtained in this experiment are presented in Table IV.

TABLE IV.—*Production of sweet-clover seed by plants protected from insect visitation during their entire flowering period at Ames, Iowa, in 1916.*

Plant.	Racemes per plant.	Pods produced by all racemes.	Average number of pods to the raceme.	Plant.	Racemes per plant.	Pods produced by all racemes.	Average number of pods to the raceme.
No. 1	84	17	0.20	No. 7	119	13	0.10
No. 2	130	58	.44	No. 8	182	83	.45
No. 3	166	30	.18	No. 9	340	142	.41
No. 4	199	88	.44				
No. 5	243	35	.14	Total	1,594	502	
No. 6	131	36	.27	Average			.31

The results given in Table IV show that an average of 0.31 of a pod to the raceme was obtained from 1,594 racemes and that the variation in seed production of the different plants was from 0.1 to 0.45 to the raceme. The average seed production for the nine plants

is 0.11 seed to the raceme less than the average results obtained from the six plants that were covered with cheesecloth. As this difference is well within the limit of variation for individual plants, it may be stated that the shading of the plants in the cheesecloth-covered cages did not reduce the production of seed. The results of this experiment show that spontaneous self-pollination does not occur regularly, as stated by Kirchner.

FLOWERS POLLINATED ONLY BY NIGHT-FLYING INSECTS.

In order to determine the importance of night-flying insects as pollinators, two cheesecloth-covered cages 3 feet square and 3½ feet high were placed over sweet-clover plants at Arlington on July 10, 1916. The covers of the cages were removed each evening at 7:30 and replaced each morning at 4:30 o'clock. Practically all the flowers on these plants had bloomed by August 2, and the seed produced was nearly mature. The few racemes that contained opened flowers or buds were discarded. The three plants in one cage produced 723 racemes, with an average of 3.76 pods each, while the one plant in the other cage produced 227 racemes, with an average of 3.58 pods to the raceme. The four plants, therefore, produced a total of 950 racemes, with an average of 3.71 pods each. The only night-flying insect found working on sweet clover while these plants were in bloom was *Diacrisia virginica* Fabr.

This experiment was duplicated at Ames in August, 1916, with the result that one plant subject to visitation only by night-flying insects produced 486 racemes, with an average of 16.5 pods each.

The results obtained in these experiments show that night-flying insects were much more active in pollinating sweet clover at Ames than at Arlington. However, as the results obtained from the plants subject to visitation by day-flying insects only were practically the same as those obtained from plants which were subject to insect visitation at all times, it is concluded that night-flying insects were not a factor in the pollination of sweet clover at Arlington or at Ames in 1916.

FLOWERS POLLINATED ONLY BY DAY-FLYING INSECTS.

A cheesecloth-covered cage, 3 feet square and 3½ feet high, was placed on July 7, 1916, over two sweet-clover plants at Arlington, before any of the flowers opened. As the cover of this cage was removed at 7.30 a. m. and replaced at 4.30 p. m. each day during the experiment, the plants were subject to visitation by day-flying insects only. As soon as all of the flowers on most of the racemes had bloomed, and before any mature pods shattered, the racemes were removed from the plants and the pods produced by each raceme counted. The two plants produced a total of 544 racemes, with an average of 20.9 pods each.

This experiment was also conducted at Ames. One plant was protected from insect visitation at night in August, 1916, with the result that it produced 418 racemes, with an average of 41.11 pods each.

PLANTS PROTECTED FROM ALL INSECTS THAT COULD NOT PASS THROUGH A WIRE SCREEN HAVING 14 MESHES TO THE LINEAR INCH.

It is well known that many small insects, and especially those belonging to the family Syrphidæ and to the genus Halictus, frequent sweet-clover flowers, but no records have been noted that show how important these insects are as pollinators of this plant. In order to obtain data on this subject a cage 12 feet square and 6½ feet high, made of wire screen having 14 meshes to the linear inch, was placed over a few plants at Ames, in July, 1916, before they began to bloom. The base of the cage was buried several inches in the soil, so that no insects could get into it. As these plants were growing in a field where there was a sufficient supply of moisture at all times, they made a growth of 5 to 6 feet. For this reason all the racemes were collected from only a portion of one of the plants instead of from the entire plant, as was done with the smaller ones inclosed in the cheesecloth-covered cages. The branches selected contained 224 racemes, with an average of 24.53 pods each. Many insects that were able to pass through the wire netting were observed working on the flowers of the inclosed plants.

A check plant, subject to visitation by all insects and growing within a few yards of the cage, contained 264 racemes, with an average of 28.23 pods each.

This experiment shows that small insects are efficient pollinators of sweet clover and that the plant to which all insects had access produced an average of only 3.7 pods to the raceme more than the one inclosed in the cage. As these plants were growing close to a strip of timber and some distance from a field of sweet clover, it is probable that more small insects worked on the flowers than would have been the case if the cage had been located in the center of a field of sweet clover. Though these results show that small insects are able to pollinate sweet-clover flowers freely, it is very doubtful whether insects of this kind would be numerous enough to pollinate sufficient flowers in a large field of sweet clover for profitable seed production. The honeybee is the most efficient pollinator of this plant, and it is believed that in many sections it is responsible for the pollination of more than half of the flowers.

SUMMARY OF INSECT-POLLINATION STUDIES.

The data secured in the different experiments where sweet-clover flowers were subject to insect visitation at one time or another are presented in detail in Table V.

TABLE V.—*Summary of the insect pollination studies conducted at Arlington, Va., and Ames, Iowa, in 1916.*

Location.	Number of plants.	Method of treatment.	Number of—		
			Racemes.	Pods produced.	Pods per raceme, average.
Arlington	1	Check—subject to insect visitation at all times.	196	4,013	20.47
Ames	1	....do	239	9,943	41.60
Arlington	3	Protected from all insects	904	577	.63
Ames	12	....do	2,370	653	.27
Arlington	3	Visited by night-flying insects only (cage 1).	723	2,720	3.76
Do	1	Visited by night-flying insects only (cage 2).	227	152	.67
Ames	1	Visited by night-flying insects only	486	8,024	16.51
Arlington	2	Visited by day-flying insects only	544	11,397	20.95
Ames	1	....do	418	17,186	41.11
Do	9	Protected from all insects	1,594	502	.31

The results in Table V show that an average of 0.37 pod to the raceme was obtained from the plants protected from visitation by all insects during the flowering period. As the racemes of *Melilotus alba* will average approximately 50 flowers each, less than 1 per cent of them set seed without being pollinated by insects. The results obtained in the cages in which only night-flying insects had access to the flowers show that these insects pollinate sweet clover to a slight extent, but that the number of pods produced by them is so few that it may be assumed that these flowers would have been pollinated by day-flying insects. This assumption is borne out by the results obtained in the cages where only day-flying insects had access to the flowers, as the results obtained in these cages at Arlington and Ames, respectively, are approximately the same as those obtained on the plants subject to insect visitation at all times. It will be noted that the yield of seed on the plants visited by insects at Ames is much higher than that of the plants subjected to insect visits during the same period at Arlington. This difference in seed yield may be attributed to the fact that isolated plants were used in the experiments at Ames, and at Arlington the experiments were conducted with plants growing under field conditions.

RELATION OF THE POSITION OF THE FLOWERS ON MELILOTUS ALBA PLANTS TO SEED PRODUCTION.

Observations of sweet-clover plants grown under cultivation, and especially when the stands were thick, showed that the flowers of the racemes on the upper and exposed branches produced a larger percentage of seed than those on the lower branches which were less exposed. It is thought by some that the failure of the flowers on the lower racemes to be fertilized is due to shading; but the results obtained in the cheesecloth and glass covered cages do not warrant this

belief, as it is doubtful whether the shading of the flowers on the lower racemes is more than that caused by the cheesecloth. It is probably the lack of pollination that causes this decrease in seed production on the lower branches of plants growing close together, as a vast number of flowers open each day on portions of the plants which are exposed directly to visitation by insects and are therefore more accessible to them.

In order to obtain information upon the number of flowers that produce seed on the upper and lower portions, respectively, of sweet-clover plants when grown under field conditions and where the stand contained four to five plants to the square foot, a number of racemes were labeled on different portions of the plants at Ames in 1915 and 1916. When the pods were partly mature, records were made of the number of flowers that produced pods. The results obtained are given in Table VI.

TABLE VI.—*Relation of the position of sweet-clover flowers on the plants to seed production, at Ames, Iowa, in 1915 and 1916.*

Year.	Position of the flowers.	Number of flowers.	Pods formed.		
			Number.	Percentage.	Average.
1915	Upper half of plants	812	357	43.9	42.6
1916	do	261	101	38.7	
1915	Lower half of plants	344	44	12.7	18.3
1916	do	216	59	27.3	

The flowers on the upper racemes of the plants produced 31.2 per cent more pods than those on the lower racemes in 1915, and 11.4 per cent more in 1916. These results prove that insects more frequently visit the flowers that are directly exposed and are therefore more accessible.

INFLUENCE OF THE WEATHER AT BLOSSOMING TIME UPON SEED PRODUCTION.

The seed production of sweet clover is seldom satisfactory when rainy or muggy weather prevails during the flowering period. In order to obtain data as to the relation existing between the visits of insects and the prevailing weather conditions, a record of insect visits and of the number of flowers that opened each day was kept for a period of nine days at Ames in August, 1915.

In this experiment the racemes were marked early each morning just above the last flowers which had opened the previous day, and early the following morning the number of flowers which had opened the previous day was noted. The number of flowers that were pollinated was determined by the number of pods that formed. Table VII gives in detail the results obtained.

TABLE VII.—*Influence of the weather at blossoming time upon the yield of sweet-clover seed, at Ames, Iowa, in 1915.*

Date, 1915.	Weather conditions.	Insect visitors.	Number of flowers that opened.	Pods formed.	Percentage of flowers that matured.
Aug. 16	Cloudy and showery	Very few	102	18	17.6
Aug. 17	Rain all day	None	69	4	5.7
Aug. 18	Cloudy most of the day	Very few	60	20	33.3
Aug. 19	Clear and cool	Numerous	94	53	56.3
Aug. 20	Mostly clear and warm	do	61	38	62.2
Aug. 21	Clear and warm	do	81	44	54.3
Aug. 22	Partly cloudy and warm	do	181	100	55.2
Aug. 23	do				
Aug. 24	Cloudy till mid-afternoon	Few	37	12	32.4

The data given in Table VII show that the percentage of effective pollination is much higher in clear weather, when insects are active, than in cloudy or rainy weather, when but few insects visit the flowers.

INSECT POLLINATORS OF SWEET CLOVER.

On account of the ease with which the heavy flow of nectar of sweet-clover flowers may be obtained many insects visit the flowers, thereby pollinating them. While the useful insect visitors of flowers of red clover are limited to a few species of Hymenoptera, those pollinating sweet-clover blossoms are many and belong to such orders as Coleoptera, Lepidoptera, and Diptera, as well as to the Hymenoptera. However, in the United States the honeybee is the most important pollinator of sweet clover. In many parts of the country the different species of Halictus, commonly known as sweat bees, rank next in importance. The margined soldier beetles (*Chauliognathus marginatus* Fabr.) were very active pollinators at Arlington, Va., in the latter part of June and first part of July, 1916, but the woolly bear (*Diacrisia virginica* Fabr.) was the only night-flying insect found working on sweet clover at Arlington.

Insects belonging to the genera Halictus, Syritta, and Paragus were very active pollinators at Ames, Iowa, in 1916, and ranked next in importance to the honeybee. In fact, the results obtained in the cage where the plants were protected from visitation by insects that could not pass through a screen having 14 meshes to the linear inch showed that these small insects were able under the conditions of that experiment to pollinate practically as many flowers as larger insects.

The insects listed below were collected while visiting *Melilotus alba* and *M. officinalis* flowers in 1916.

AT ARLINGTON, VA.

Neuroptera.—*Perithemis domitia* Dru., *Enallagma* sp.

Hemiptera.—*Adelphocoris rapidus* Say, *Lygus pratensis* Linn. (tarnished plant bug).

Coleoptera.—*Chauliognathus marginatus* Fabr. (margined soldier beetle), *Diabrotica 12-punctata* Oliv. (southern corn rootworm).

Lepidoptera.—*Pieris protodice* Bd. (imported cabbage butterfly), *Heodes hypophleas* Bd., *Lycaena comyntas* Gdt., *Hylephila campestris* Bd., *Scepsis fulvicollis* Hubn., *Ancyloxypha numitor* Fabr., *Pholisora catullus* Fabr., *Pyraustid* sp., *Loxostege similalis* Gn. (garden webworm), *Thecla melinus* Hubn., *Colias philodice* Gdt. (the common sulphur butterfly), *Tarachidia caudefactor* Hubn., *Pyrameis atalanta* Linn., Drasteria (2 species), *Diacrisia virginica* Fabr. (the woolly bear).

Hymenoptera.—*Halictus lerouxi* Lep., *H. provancheri* (sweat bee), *H. pectoralis* Sm. (sweat bee), Halictus (3 unidentified species), *H. legatus* Say, *Bombus affinis* Cr., *B. impatiens* Harris (bumblebee), *Melissodes bimaculata* Lep., *Polistes pallipes* Lep. (paper wasp), *Megachile* sp. (leaf-cutter bee), *Coelioxys octodentata* Say, *Xylocopa virginica* Drury (common carpenter bee), *Pompiloides* sp., *Apis mellifica* Linn. (honeybee), *Philanthus punctatus* Say, *Sphex nigricans* Dahlb. (caterpillar hawk), *S. pictipennis* Walsh (caterpillar hawk).

Diptera.—*Archytas analis* Fabr., *Chrysomyia macellaria* Fabr. (screw-worm fly), *Pollenia rudis* Fabr. (cluster fly), *Ocyptera carolinae* Desv., *Trichophora ruficauda* V. D. W., *Eristalis arbustorum* Linn., *Physocephala tibialis* Say.

AT AMES, IOWA.

Hemiptera.—*Lygus pratensis* Linn., *Adelphocoris rapidus* Say.

Coleoptera.—*Coccinella transversoguttata* Fabr.

Lepidoptera.—*Eurymus eurytheme* Bdv., *Chrysophanus* sp., Lycaena (2 species), *Libythea bachmani* Kirtland, *Pieris rapae* Linn.

Hymenoptera.—*Angochlora* sp., *Apis mellifica* Linn., *Colletes* sp., *Halictus lerouxi* Lep., *H. provancheri* D. J., *Halictus* sp., *Elis* sp., *Calliopsis andrêniformis* Smith, *Polistes* sp., *Sphex* sp., *Eumenes fraterna* Say, *Sceliphron* sp., *Isodontia harrisi* Fern., *Cerceris* sp., *Oxybelus* sp.

Diptera.—*Syritta* sp., *Paragus* sp., *Chrysomyia macellaria* Desv., Syrphidæ (2 unidentified specimens).

EFFECT OF MOISTURE UPON THE PRODUCTION OF MELILOTUS ALBA SEED.

Careful inspection of a number of sweet-clover fields in Iowa and Illinois in the autumn of 1916 indicated that many plants were unable to obtain sufficient moisture for the proper development of their flowers. Examination of flowers that aborted shortly after reaching their mature size showed that the anther sacs had not burst, even though the pollen grains were mature. Apparently for the same reason many immature pods aborted. The precipitation for July, 1916, in Livingston County, Ill., where the sweet-clover seed crop suffered materially for lack of moisture, was 3.2 inches less than normal, while the temperature was 4.5° F. above normal. In August the precipitation was 0.96 of an inch below normal and the temperature 4.2° F. above normal. At Ames, Iowa, the precipitation was 3.54 inches below normal and the temperature 5.4° F. above

normal in July. Both the precipitation and temperature were about normal at Ames in August, but most of the precipitation fell before the experiments were commenced.

In north-central Illinois the seed production of sweet clover was very irregular. Some fields produced an abundance of seed, while a large percentage of the pods on the plants in other fields near by, where the thickness of the stand, size of the plants, and conditions in general were approximately the same, aborted. It was evident that all stands producing a good seed crop were growing on well-drained soil and that those which were not yielding satisfactorily were on poorly drained land. It is well known that sweet clover will produce deep taproots only when the plants are growing in well-drained soil and that a much-branched surface root system will be formed on poorly drained land, and especially when there is an excess of moisture or a high water table during the first season's growth. During this droughty period in 1916 the upper layer of soil became so depleted of moisture that the plants with surface root systems were unable to obtain sufficient water to mature their seed. On the other hand, the lack of precipitation and the high temperatures did not affect the moisture content of the subsoil sufficiently to interfere with the normal seed production of deep-rooted plants. According to Lutts (22, p. 47) this same condition was found to be true in Ohio in 1916.

As a rule, under droughty conditions the second crop of sweet clover will produce a higher yield of seed than the first crop, as the second growth of the plants is seldom more than half as much as the first, thereby requiring less moisture. However, if showery hot weather prevails when the first crop is cut, the end of each stub is very apt to become infected, usually with a species of Fusarium, which kills all the cortex as far back as the upper bud or young shoot and that part of it on the opposite side of this bud to the bud below. If the second bud from the top of a stub is not directly opposite the upper one the decay may extend nearly to the ground. (Pl. IV.) The destruction of half to two-thirds of the cortex from 2 to 4 inches below the upper bud materially reduces the quantity of water that can be conveyed to the branch above the base of the dead area. Plants thus infected obtain sufficient moisture for seed production only under the most favorable conditions. When the first crop is cut during warm dry weather, and especially when the first crop has not been permitted to make more than a 30 to 32 inch growth, the stubble seldom decays, and in no instance have the plants been observed to decay as far back as the upper buds.

An experiment was conducted at Ames in the latter part of August and first part of September, 1916, to determine the effect of watering plants that were aborting a large percentage of their flowers and

immature pods. For this purpose several volunteer plants growing in a meadow were selected. A hole 12 inches square and 14 inches deep was dug 8 inches from the crown of one plant, and each evening during the experiment 2 gallons of water were poured into the hole. The top of the hole was kept covered, so as to check evaporation from it as much as possible. Another plant of the same size and growing about 15 yards from the watered plant served as a check. On both plants many of the flowers and immature buds were aborting at the beginning of the experiment. The soil in this field was so depleted of moisture that the leaves of the plants wilted during the hottest part of the days preceding the experiment. The foliage on the check plant wilted each day for the first five days of the experiment. On the sixth day 0.96 of an inch of rain fell and four days later 0.23 of an inch more. The dropping of the flowers was temporarily checked by these precipitations, but owing to the dry, compact condition of the soil the rain was not sufficient to check entirely the fall of flowers and immature pods. At the beginning of the experiment the racemes on both plants were divided into three classes, according to the development of the flowers, and labeled. They were collected and the seeds counted as soon as the pods at the bases of the racemes commenced to turn brown. Table VIII presents the results obtained.

TABLE VIII.—*Effect of water upon the seed production of sweet clover when growing under droughty conditions at Ames, Iowa, in 1916.*

Stage of development when labeled.	Plant not watered.		Plant watered.		Increase from watering.
	Number of racemes labeled.	Average number of pods per raceme that matured.	Number of racemes labeled.	Average number of pods per raceme that matured.	
Flowers at the base of the racemes just ready to open	49	27.39	110	55.63	28.24
Pods 3 to 6 days old	30	21.13	112	39.81	18.68
Pods 9 to 12 days old	35	15.23	50	29.86	14.63

The effect of the water was noticeable soon after the first application, as the leaves and flowers on this plant became turgid and the anther sacs burst at the proper stage of their development. Very few flowers fell after the second day. The water decidedly checked the aborting of immature pods, as is shown by the results obtained on the racemes which were labeled after the pods had formed. The racemes which contained pods 3 to 6 days old when labeled matured 9.95 pods to the raceme more than those which contained older pods at the beginning of the experiment, but this was expected, as most of the aborting which caused this difference had taken place before the racemes were labeled. As very few pods aborted before they were 3 to 6 days old, the difference of 9.95 pods to the raceme in favor

of the ones labeled when the flowers at their bases were just ready to open was largely due to the dropping of the flowers on the older racemes before the experiment was begun.

It will be seen that the production of mature pods on the plant not watered was much greater on the racemes that were labeled before the flowers opened than on the older racemes. This difference is undoubtedly due to the precipitation which fell on the sixth and tenth days of the experiment. It is believed that the yield of 15.23 pods to the raceme on the ones labeled when the pods were 9 to 12 days old is representative of the production of pods per raceme previous to the precipitation and that the other racemes on this plant would have yielded proportionately if conditions had remained the same.

In the early spring of 1916, *Melilotus alba* was planted in several large pots in the greenhouse of the Department of Agriculture at Washington, D. C. These pots were placed outside the greenhouse in the late spring, where they remained until the following January, when they were taken into the greenhouse. The plants grew rapidly and began to flower during the latter part of April, 1917. At this time two pots were placed in a large cage made of screen having 20 meshes to the linear inch. One pot was submerged in a tub of water, so that the soil was saturated at all times, while the plant in the other pot was given only sufficient water to keep it from wilting. The pods on a few racemes were self-pollinated and the results obtained are given in Table IX.

TABLE IX.—*Effect of moisture on the seed production of Melilotus alba at Washington, D. C., in 1917.*

Soil treatment.	Total number of—			Flowers that matured (per cent).	
	Racemes.	Flowers.	Pods formed.	Total.	Increase.
Soil given only a limited quantity of water	12	227	65	28.63	.....
Soil saturated	17	425	234	55.05	26.22

The results of this experiment compare favorably with those obtained under field conditions at Ames in 1916.

Part II.—STRUCTURE AND CHEMICAL NATURE OF THE SEED COAT AND ITS RELATION TO IMPERMEABLE SEEDS OF SWEET CLOVER.[1]

HISTORICAL SUMMARY.

When agriculturists first began to cultivate wild legumes they observed that many seeds would not germinate within a comparatively short time after planting. Thus the problem of impermeable seeds began to demand attention many years ago. However, impermeable seeds are not confined to the Leguminosæ, as they occur also in the Malvaceæ, Chenopodiaceæ, Convolvulaceæ, Cannaceæ, and other families.

Since the first account of the structure of legume seed coats by Malpighi (23 v. 1) in 1687, many investigators have contributed to our knowledge of the structure of the coats of seeds belonging to this family.

Pammel (31) made an extensive study of legume seeds, including all the genera in the sixth edition of Gray's Manual, as well as genera not included in that publication. He found that the seed coat uniformly consisted of three layers, namely, the outer layer of Malpighian cells, the osteosclerid layer, and the inner layer of nutrient cells. Pammel's work included a study of the seed coats of *Melilotus alba* and *M. officinalis*, and the descriptions and illustrations in his publication agree for the most part with the results obtained in the investigations reported in this article. However, more variation was noticed in the different layers of the seed coat than he describes.

The cause of impermeability in seeds has been investigated by many. It has been found to be due to the embryos in some seeds, such as the hawthorns, but in most cases to the structure of the seed coat, and especially so in the Leguminosæ. Crocker (3) states that, exactly opposite to the common view, the cause of delayed germination generally lies in the seed coats rather than in the embryos. Nobbe (29) thought that the hardness of leguminous seeds was due to the Malpighian layer, and in a later publication Nobbe and Haenlein (30, p. 81) state that the absorbent power of many seeds is inhibited or entirely arrested by the cones of the Malpighian cells and the shields built up between them, which consist principally of cutin. Huss (15) agrees with Nobbe and Haenlein. Verschaffelt (39) found that the impermeability of the seeds of Cæsalpiniaceæ and Mimosaceæ investigated was due to the inability of water to pass through the canals of the seed coat. By soaking the seeds in alcohol or other substances which change the capillarity of the pores, the seed

[1] The writers wish to acknowledge the service rendered by Mr. H. S. Doty, Instructor in Botany, Iowa State College, Ames, Iowa, in assisting in the preparation of this article.

coats were made readily permeable to water. Gola (6) states that the cause of the impermeability of seeds is the peculiar character of the Malpighian cells, which prevents their infiltration and consequent increase in volume, while Bergtheil and Day (2) found that the hardness of the seeds of *Indigofera arrecta* was due to their possession of a very thin outer covering of a substance resistant to water. Ewart (5, p. 185) believes that in most impermeable seeds the cuticle prohibits the absorption of water, but gives as an exception *Adansonia digitata*, in which the whole integument seems to be permeable to water with difficulty. The following is quoted from White (42, p. 205):

> As a general rule in small and medium-sized seeds the cuticle is well developed and represents the impermeable part of the seed coat, while in the cases of large seeds, such as those of *Adansonia gregorii*, *Mucuna gigantea*, *Wistaria maideniana*, and *Guilandina bonducella*, the cuticle is relatively unimportant and inconspicuous. In these seeds the extreme resistance which they exhibit appears to be located in the palisade cells.

In discussing the seed coat of *Melilotus alba*, Rees (33, p. 404) states that the outer layer consists of palisade cells covered externally by a structureless membrane, which, however, did not appear to be cuticle but hemicellulose, as it stained magenta with chloriodid of zinc. The greater part of the walls of the palisade cells also appears to be composed of hemicellulose and the outer ends only were cuticularized. In order to find whether the outer membrane was in itself impermeable to water, this author treated seeds for short intervals in sulphuric acid to dissolve the outside covering without directly affecting the palisade cells. Seeds treated in this manner swelled in water and microscopic examination showed that the ends of the palisade cells were quite intact, but had separated from each other. From this it was concluded that the outer membrane is instrumental in conferring impermeability on the seed, although not directly responsible for it, as is the case with a true cuticle. It is further believed that it probably served as a cement substance by means of which the cuticularized ends of the cells were held together closely, thus forming a barrier through which water could not penetrate, but that as soon as this barrier was removed the ends of the palisade cells separated and water passed in between them.

More than 20 years ago machines were devised by Kuntze, Michalowski (27, p. 86), Huss (15), and later by Hughes (14), to scarify impermeable seeds. Other methods have been recommended and employed to some extent for hastening the germination of seeds. Hiltner (13, p. 44) treated seeds of red clover, white clover, and alfalfa 10, 30, and 60 minutes with concentrated sulphuric acid and found that the best germination resulted from the 60-minute treatment. Love and Leighty (21) also treated the seeds of various

legumes with concentrated sulphuric acid and obtained a better germination in all cases. In their investigations with *Melilotus alba* it was found that a 2-hour treatment resulted in some injury to the seed, but that a treatment varying from 25 minutes to 1 hour gave good results. In most cases in our investigations the seed coats of sweet clover became permeable to water after a treatment of 15 minutes in concentrated sulphuric acid, and within 5 minutes all of the Malpighian cells were destroyed down to the light line. Harrington (10) found that the soil, season, climate, color, or size of red-clover seeds had no influence upon the percentage of impermeable seeds and that the good germination ordinarily obtained with red clover was due to the scarifying of the seed coats by the rasps of hulling machines. Harrington (11) also studied the agricultural value of impermeable seeds and found that alternations of temperature cause the softening and germinating of many impermeable clover seeds when a temperature of 10° C. or cooler is used in alternation with a temperature of 20° C. or warmer and that the effect of such an alternation of temperature is greatly increased by previously exposing the seeds to germinating conditions at a temperature of 10° C. or cooler and is decreased by previously exposing the seeds to germinating conditions at a temperature of 30° C. It is a well-known fact that impermeable seeds which remain in the field over winter germinate readily the following spring.

The light line is the most important and interesting feature of the Malpighian cell, at least so far as *Melilotus alba* and *M. officinalis* are concerned. But one light line occurs in the Malpighian cells in most Leguminosæ, although Pammel (32) reports two well-developed light lines in *Gymnocladus canadensis*, Junowicz (16) found three in *Lupinus varius*, and Sempolowski (36) two in *Lupinus angustifolius*.

Many investigators have studied the light line, and different theories have been advanced as to its function, physical properties, and chemical nature. Schleiden and Vogel (35, p. 26) in describing the mature testa of *Schizolobium excelsum* in 1838 undoubtedly referred to the light line when they stated that the walls of the Malpighian cells were not equally thickened. Mettenius (26), in 1846, was probably the first definitely to describe the light line. This author believed it was composed of pore canals, all appearing at the same height in the cells, but he was unable to prove this by cross sections. Lohde (20) studied the light line in seeds of *Hibiscus trionum* and found it cutinized. Hanstein (8) states that the Malpighian cells are composed of two cell layers and the light line is produced by the adjoining walls of the ends of the cells. Later, this same author (9), according to Harz (12), refers to the light line as a perforated disk composed of tissue of strong refracting power.

Russow (34) concludes that the light line is produced by neither chemical nor mechanical changes but is caused by a modified molecular structure containing less water than the remainder of the cell wall. Hiltner (13) agrees with Russow's explanation. Harz (12, p. 561) also agrees with Russow and adds that he has observed that the light line disappeared in a number of cases after applications of nitric acid. Wigand and Dennert (43) suggested that the light line is due to a series of erect fissures, while Tietz (37, p. 32) believes it is due to a chemical modification and that the phenomenon results from the exceptionally extreme density of parts of the cellulose membrane. Junowicz (16) found evidence of cellulose material. The cell wall at this point was strongly refractive and had a different molecular structure. After studying *Phaseolus vulgaris*, Haberlandt (7, p. 38) agrees with the Russow explanation. In the seed of this plant the light line colored blue after being treated with chloriodid of zinc. Sempolowski (36), who investigated the light line in *Lupinus angustifolius*, states that there is not only a difference in the molecular structure but also a chemical modification of the cell wall at this point, since with iodin and sulphuric acid the cell wall colored blue, whereas the light line colored yellow. Wettstein (41), who studied seeds of Nelumbo, agrees with Russow (34) and Sempolowski (36) that chemical and physical modifications occur. He found that iodin and sulphuric acid colored the Malpighian cells intensely blue, the light line at first yellowish, and then later it gradually became blue. This reaction may be accelerated by heat. Iodin produced the same effect, and the light line colored blue more rapidly. When treated with a water-withdrawing medium the light line was not altered for some time, but finally disappeared with continued application. Cooking for a long time in caustic potash or standing in cold caustic potash caused the cells to swell, while the light line remained uninjured at first but finally disappeared. He also believed that the absence of pore canals in the region of the light line caused it to be more dense.

Nobbe and Haenlein (30) treated sections of seed coats of *Trifolium pratense* with iodin and sulphuric acid and found that the light line colored blue as readily as the thickened ridges that radiate inward from it, but that the outer processes of the palisade cells projecting from the light line toward the cuticle stained dark brown. They also state that various causes work to produce such unusual lusters in the light line, the principle one of which is the thickened ridges which radiate inward, reach their greatest development at this point, and coalesce in the lumen of the cell. The result is that the light line falls upon a continuously homogeneous medium, while in the inner portions of the ridges the light passes through media of varying opacity, such as cellulose, water, and protoplasm, whereby it is pro-

gressively subdued in varying degrees by partial reflection. Pammel (31, p. 147) studied the light line in *Melilotus alba* and found that it consisted of a narrow but distinct refractive zone below the conical layer. The refractive zone colored blue with chloriodid of zinc. The whole upper part was, however, more or less refractive, while the remainder of the cell wall contained pigment and colored blue with chloriodid of zinc. Small canals project into the walls, in some cases extending beyond the light line.

Beck (1) found that the light-refracting power of the light line was much greater than that of the undifferentiated membrane and stated that there may be in addition to this a chemical difference which can not be detected with the present microchemical methods. He does not believe that it is cuticularized or that it contains less water than the rest of the cell.

Marlière (24, p. 11) gives a physical explanation and states that the true cause of the light line lies in the peculiar structure of the secondary membrane of the Malpighian cell. Tunmann (38, p. 559) observed that it did not hydrolize in weak acids and therefore decided that it was not hemicellulose. He found that it dissolved in concentrated sulphuric acid more readily than the regions surrounding it and that it was composed of pectin or callose. In our investigations the main portion of the light line of *Melilotus alba* and *M. officinalis* was very resistant to concentrated sulphuric acid, only the narrow outer portion being attacked. It showed evidence of callose.

MATERIAL AND METHODS.

Permeable and impermeable seeds[1] of *Melilotus alba* and *M. officinalis* were obtained from commercial samples and also from samples collected in the field. Those selected for sectioning were allowed to dry after being removed from the germinator and then embedded on the ends of pine blocks in glycerin gum, which was made by dissolving 10 grams of powdered gum arabic in 10 c. c. of water and adding 40 drops of glycerin. After the glycerin gum had dried for 24 hours, the seeds were easily sectioned. This method of embedding causes no change in the seed coat. It is more satisfactory than the paraffin method for holding the seeds firmly. The glycerin gum dissolved readily when the sections were mounted in water.

In the microchemical studies Sudan III, alcanin, chlorophyll solution, and phosphoric acid iodin were used to test for cutin or suberin; sulphuric acid and iodin, chloriodid of zinc, and chloriodid of calcium for cellulose; phloroglucin and hydrochloric acid for lignin;

[1] The term "permeable" is used in this paper to designate seeds whose coats are permeable to water in two weeks or less at temperatures favorable for germination, while the term "impermeable" is used to designate seeds whose seed coats are impermeable to water for this length of time when temperatures are favorable for germination. Impermeable seeds are commonly referred to as "hard seeds," and they may become permeable in time.

ruthenium red for pectic substances; and sulphuric acid, Congo red, and aniline blue for callose.

Where very thin sections were necessary for detailed study of the structure of the seed coat, pods in various stages of development were collected, and after the usual preliminary treatment they were embedded in paraffin and sectioned with the microtome. Microchemical tests were made with these sections by using various specific stains. Safranin was used to test for cutin, suberin, and lignin; haematoxylin and methyl blue for cellulose; methylene blue, methyl violet B, mauvein, and ruthenium red for pectic substances; and aniline blue and Congo red for callose. In studying some points with reference to the pore system of the seed coat, it was necessary to use free-hand sections of fresh pods.

In studying the seed coat in relation to the absorption of water, both permeable and impermeable seeds were soaked in water solutions of safranin, gentian violet, eosin, and haematoxylin, then dried and embedded in glycerin gum for sectioning. Seeds were soaked in stains dissolved in 95 per cent alcohol to test the penetration of alcohol. It was evident that the seed coats did not act as a filter, as the stains passed through them with the water or alcohol.

STRUCTURE OF THE SEED COAT.

There is very little endosperm present in mature seeds of *Melilotus alba* or *M. officinalis*. That which is present is quite permeable to water and therefore bears no relation to the impermeable seeds of these plants.

The outer layer of the seed coat, which is the modified epidermal layer of the ovule, is known as the Malpighian layer. (Pl. V, figs. 1 and 2.) The cells constituting this layer, commonly called palisade cells, are the most highly modified cells of the seed coat. They are very much elongated, their length varying in the different regions of the coat, and their outer tangential walls and the outer portions of their radial walls are so much thickened that their lumina are confined to the inner portion of the cells, sometimes occupying less than half the length of the cells. The inner tangential walls and inner portions of the radial walls are thickened just previous to the death of the cells, the thickening sometimes being only slight and sometimes so much as to leave only very narrow lumina.

There is a very thin layer on the outer surface of the Malpighian cells which has been called cuticle by previous investigators, but the chemical composition of this layer and its perviousness to water indicate that there is very little cutin present. This layer is probably the primary epidermal cell wall rather than a deposit on the outer surface of the wall. To determine this a study of the development of the Malpighian cells is necessary.

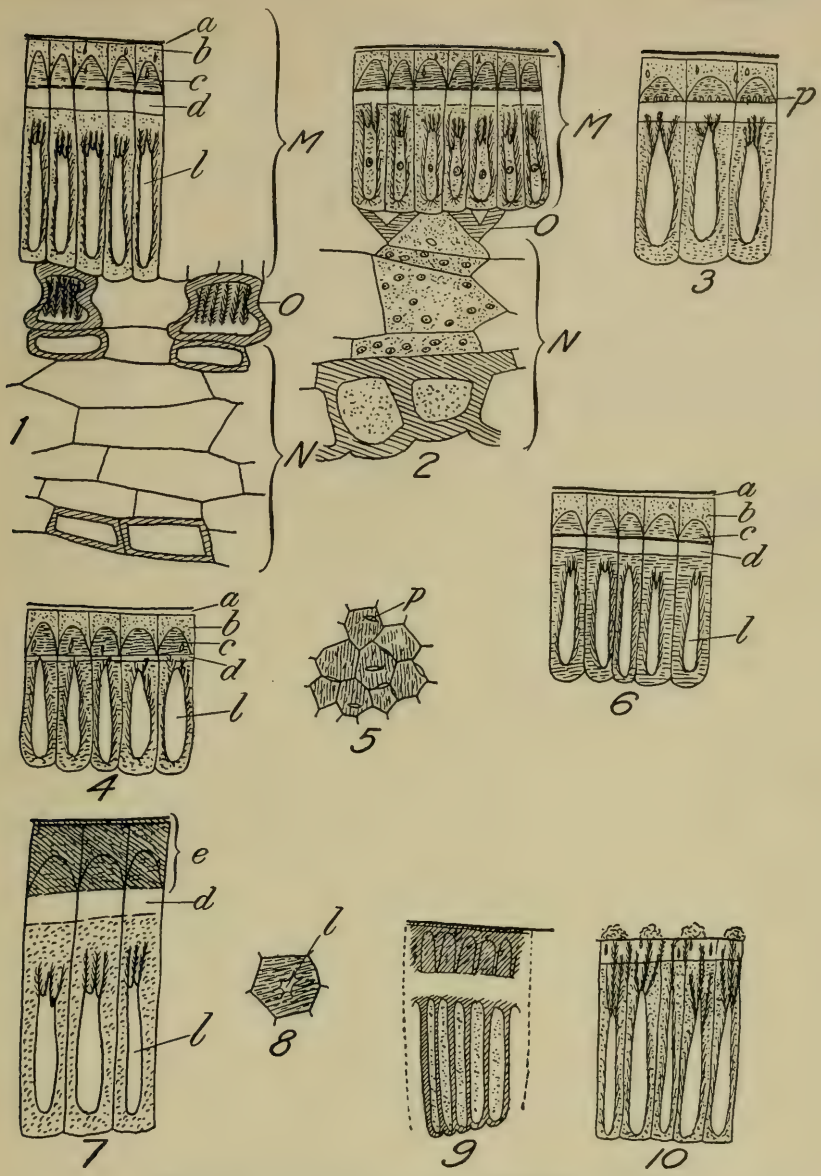
Beneath the so-called cuticle there is the much thickened outer portion of the Malpighian cells in which there are two rather distinct regions, one constituting the conelike structures and the other forming a continous layer over the conelike structures, separating them from the cuticle and filling in between them. These two regions separate easily, and in cutting sections the outer region, called by some the cuticularized portion, often breaks away, leaving the entire surface of the cones exposed.

The term "cuticularized layer" will be used to designate all of the thickening covering the cones, including that around the cones as well as the portion between the cones and the cuticle. This term is not entirely appropriate, for the region is practically free from cutin, but for the want of a better term it will be used. There are canals in the cuticularized layer and cones, which are easily seen when the sections are treated with chloriodid of zinc or sulphuric acid. A surface view of a section showing the cones and cuticularized layer when mounted in glycerin shows the canals as dark lines due to the air inclosed. The canals are most abundant along the lines where the lateral walls of the cells join, but many are within the cones and in the cuticularized substance between the cones. (Pl. V, fig. 5.)

The well-developed light line in *Melilotus alba* and *M. officinalis* is found just below the bases of the cones. In some seed coats only a few and in others none of the canals which are common in the cones and cuticularized region cross the light line. A very distinct line of small canals filled with air and thus forming a dark band is present just above the light line, thus making the light line more conspicuous. (Pl. V, fig. 3.) When the lumina of the cells extend across the light line, they are exceedingly small. The light line is the most compact region of the Malpighian layer and is conspicuous because it refracts the light much more than the regions above and below it.

Just below the Malpighian is a layer of cells variously modified and known as the osteosclerid. The cells of this layer are often referred to as the hourglass cells on account of their shape. In some regions of the seed coat they are expanded at both ends and their walls are much thickened, the thickenings forming ridges on the radial walls, while in other regions only the upper tangential wall and a portion of the radial walls are thickened and the cells are expanded only at the inner end, thus having the shape of the frustum of a cone. Beneath the osteosclerid layer is the nutrient layer.

The nutrient layer contains chloroplasts. It varies not only in the number of layers of cells composing it, but also in the modifications of these cells. This layer ranges from four to seven cells in thickness in the different parts of the seed coat.



STRUCTURE OF THE SEED COAT OF SWEET CLOVER.

FIG. 1.—Section of the seed coat of *Melilotus officinalis*. ×450. FIG. 2.—Another section of the seed coat of *Melilotus officinalis*, showing the variation in size and modifications that occur in the three layers. ×450. FIG. 3.—Section of the Malpighian layer of a *Melilotus alba* seed, showing a line of canals just above the light zone. ×450. FIG. 4.—Section of the Malpighian layer of a permeable *Melilotus alba* seed. ×450. FIG. 5.—Tangential section of the Malpighian cells cut between the cuticle and tops of the cones, showing pores. ×530. FIG. 6.—Section through the Malpighian layer of an impermeable *Melilotus alba* seed. ×450. FIG. 7.—Section through the Malpighian layer of an impermeable *Melilotus alba* seed, showing the region through which water and stains readily passed. ×450. FIG. 8.—Cross section of a Malpighian cell of a permeable *Melilotus alba* seed through the region of the light zone, showing the lumen not entirely closed. ×530. FIG. 9.—Section through the Malpighian layer of a *Melilotus alba* seed shaded to show the portions which react to the cellulose and pectose tests. ×450. FIG. 10.—Section through the Malpighian layer of a *Melilotus alba* seed which shows the condition of the seed coat after 60 minutes' treatment of concentrated sulphuric acid. That portion above the light zone was destroyed, and the lumina as small pores through which much of the stain now passed were seen extending across the light line. The lines between the cells were much more distinct, appearing as small intercellular spaces through which some stain passed. ×450. *a*, Cuticle; *b*, cuticularized layer; *c*, conelike portion of the thickening of the Malpighian cells; *d*, light line; *e*, region of a hard seed coat through which water and stains readily passed; *l*, lumen; *M*, Malpighian cells; *N*, nutrient cells; *O*, osteosclerid cells; *p*, canals just above light zone.

MICROCHEMISTRY OF THE SEED COAT.

Tests for cutin showed that there was very little present in the seed coat. Slight reactions for cutin were observed in the cuticle, in the outer margin of the cuticularized layer, and in the basal portion of the cones. These reactions were so slight as to be almost negligible. It is evident that the cuticle and cuticularized layer are not well named in *Melilotus alba* and *M. officinalis*. Tests for cellulose showed that it was present in the cuticle, cuticularized layer, cones, the walls of the Malpighian cells below the light line, and the walls of the cells of the osteosclerid and nutrient layers. (Pl. V, fig. 9.) The reaction for cellulose in the Malpighian cells was quite distinct in the walls below the light line, less distinct in the cones and cuticle, and least distinct in the cuticularized layer.

Tests for lignin occasionally showed slight traces in the Malpighian cells below the light line. When treated with reagents for pectic substances, the cuticle, cuticularized layer, cones, and all cell walls below the light line gave a definite reaction. The reaction of the cones and cuticle was more pronounced than the cuticularized layer. Tests for callose gave no reaction except in the upper part of the light line. This part of the light line stained slightly blue with aniline blue and was easily dissolved with sulphuric acid. In cutting free-hand sections of fresh material the Malpighian layer sometimes broke along this line. The greater part of the light line reacted to none of the tests, and its chemical nature was not determined.

When microtome sections of seeds in different stages of development were treated with various stains, the results were in accord with those obtained with free-hand sections. Thus with safranin the periphery and cones of the Malpighian cells were slightly stained, while haematoxylin and methyl blue stained all the seed coat except the light line. The cones and cuticle stained more readily than the cuticularized layer, but neither stained as deeply as the cell walls below the light line. Methylene blue, methyl violet B, and mauvein stained all above the light line, indicating the presence of pectic substances; however, the staining was more prominent in the cones and cuticle.

The difference in reaction of the cones and cuticularized layer to the cellulose and pectose tests probably indicates a difference in density rather than a difference in chemical composition. Since the cuticularized layer separates readily from the cones, there may be a difference in physical properties.

With Congo red the upper part of the light line was only very slightly stained, but aniline blue had a more pronounced effect.

The microchemical tests applied to the seed coat show that in the region above the light line there is only a slight trace of cutin or

suberin, but a considerable amount of cellulose and pectose. All cell walls below the light line are mainly cellulose but contain some pectose. The upper portion of the light line contains callose, but the remainder of the light line appears to be chemically different from all other parts of the seed coat or else so dense as to resist the attack of the reagents.

THE SEED COAT IN RELATION TO THE ABSORPTION OF WATER.

A study of permeable seeds soaked in water containing stains showed that there were no local regions through which the water passed. The stains passed through all regions of the seed coat. Coating the micropylar region with vaseline retarded germination, but had no effect upon the percentage of germination at the end of three days. In seed coats through which the stain had passed, the light line was not stained. Some stain was found in the canals which crossed the light line, and much more in the cell cavities. There was no evidence that the stain had permeated the substance of the light line. It was able to cross the light line only when pores were present.

In impermeable seeds the stains passed readily to the light line. (Pl. V, fig. 7.) It was evident that the absorption of water was not prevented by either the cuticularized layer or the cone-shaped structures of the Malpighian layer, but by the light line. The region outside of the light line became stained in a few hours, but there was no trace of the stain within the light line after the seeds had remained a week in the stains. Alcohol did not penetrate the seed coat more readily than water.

A COMPARISON OF PERMEABLE AND IMPERMEABLE SEED COATS.

No difference in chemical structure was found between the coats of permeable and impermeable seeds. The principal differences were in the character and amount of thickening of the cell walls.

In many of the permeable seeds some of the canals were found to extend across the light line, but this was not true for all permeable seeds. (Pl. V, fig. 8.) Oblique sections of permeable seed coats showed that the cell cavities, although reduced to mere pores by the thickening of their radial walls, extended across the light line into the base of the cones, thus forming a passageway through which the stains passed to the larger portions of the cell cavities below the light line. (Pl. V, fig. 4.)

In the coats of the impermeable seeds the light line was usually broader, the Malpighian cells thickened more below the light line, and the main cavities of the Malpighian cells were more reduced and farther below the light line than in the coats of permeable seeds. (Pl. V, fig. 6.) No canals except occasionally a few very small ones were seen crossing the light line in impermeable seeds. Cross and

oblique sections showed that the lumina of the Malpighian cells were closed in the region of the light line. Thus it was found that permeable and impermeable seeds differ mainly in the amount of thickening which occurs in the walls of the Malpighian cells. In the impermeable seeds the thickening which begins at the outer tangential wall of the Malpighian cell extends farther toward the inner tangential wall, leaving the cell lumina smaller and farther below the light line than in permeable seeds. The thickening is also more complete in impermeable seeds, leaving fewer and smaller canals across the light line as well as closing the cell lumina in the region of the light line.

THE ACTION OF SULPHURIC ACID ON THE COATS OF IMPERMEABLE SEEDS.

Impermeable seeds were soaked in concentrated sulphuric acid (sp. gr. 1.84) for 15, 30, and 60 minutes; then washed and put in the staining solutions. After they had swollen, they were removed from the staining solutions, dried, and embedded in glycerin gum. A study of these seeds showed that the acid had eaten away all of the material outside of the light line and that the stain had passed through all regions of the seed coat. (Pl. V, fig. 10.) When observed under the microscope, it was seen that the action of the acid was rapid, destroying the cuticle, cuticularized layer, and cones in about 5 minutes. After 15 minutes treatment with acid the light line, aside from the presence of canals and pores not previously visible, seemed to be very little affected. The division lines along which the lateral walls of the Malpighian cells were joined now became much more distinct across the light line, thus indicating that there was some swelling in this region. When a close examination of the path of the stain was made the cell lumina, and occasionally very small pores, were found to extend across the light line. The presence of the stain in the pores indicated that they were paths of the stain across the light line. Some of the stain passed along the lines between cells and through the occasional canals crossing the light line, but judging from the intensity of the stain in the lumina the canals appeared to be the principal passageways.

The action of the acid in opening the cell cavities across the light line was not determined. It may be due to the swelling of the light line or to the removal of substances closing the pores.

No seeds were exposed to the acid for longer than an hour, but at the end of this period the light line was still intact. As compared with other portions of the Malpighian layer, it is extremely resistant to concentrated sulphuric acid. Since all cell walls below the light line are mainly cellulose, the resistance of the light line prevents the acid from destroying the entire seed coat and reaching the embryo.

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